Applications of hormesis in toxicology, risk assessment and chemotherapeutics

Edward J. Calabrese and Linda A. Baldwin

There is much debate over the fundamental shape of the dose–response curve in the low-dose zone, particularly in the fields of toxicology and risk assessment. The defaults, principally accepted dose–response models in the major texts in these areas and in government regulatory activities, are a threshold model for non-carcinogens and a linear model for most carcinogens. We have argued that in properly designed studies the U-shaped hormetic response predominates and is more fundamental. In this article, a broad range of basic issues associated with the acceptance of U-shaped dose responses as central to toxicology, pharmacology and their applications to risk assessment and medicine will be discussed.

PRINCIPLES

In a recent issue of *TiPS* we assessed the concept of hormesis in toxicology and pharmacology, together with the historical foundations, definitional dimensions and frequency within the toxicological literature of hormesis and its role in the assessment of the dose response in the low-dose zone and principal risk assessment and chemotherapeutic implications [1]. The present article is designed to extend that earlier effort by exploring selected areas of crucial importance to the concept of hormesis. The article is framed by a series of questions and answers that lead to a focused and fuller exploration of this topic.

Dose–response models

The field of toxicology has been dominated by the use of two dose–response models, the threshold and linear models. These two models essentially determine strategies for animal model selection, study design (including the number and range of doses employed) and the means to estimate population-based risks. This dominance has been manifest since the 1930s in the case of the threshold model and since the early 1970s in the case of the linear model approach when it was applied to assessing risks to carcinogens. These approaches have now become institutionalized in the USA by various federal and state regulatory and public health agencies along with significant worldwide influence. Not only has acceptance of these models affected how regulatory and public health agencies act, but it has also affected how professionals frame their thoughts and strategies for studying dose–response relationships. This is seen in the content of major texts on toxicology and in the major societies of toxicology where essentially only threshold and linear dose–response models are discussed.

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The principal aim of this article and its predecessor in this journal [1] is that we believe that the most fundamental, common and generally applicable dose–response model is the hormetic U-shaped biphasic model rather than the threshold and linear models. The hormetic model can be characterized by a low-dose stimulation, high-dose inhibition. The quantitative features of the hormetic dose response is a modest stimulation at low doses where the maximum stimulation is typically 30–60% greater than the controls, and a range of stimulation that can be variable but is typically less than 10–20-fold, although ~5–7% of hormetic dose responses can exceed 100-fold.

The reasons why the field of toxicology rejected the concept of hormesis are complex [2–6] but involve a mix of limitations of the hormetic model itself along with difficulties in the replication of modest low-dose stimulatory responses, lack of appreciation of the biomedical significance of low-dose stimulatory doses, close historical association with the medicinal practice of homeopathy, strong historically important opponents such as A.J. Clark, the prestigious pharmacologist, and poor scientific leadership. This combination proved too much to overcome in the early 1930s and hormesis was soon relegated to the status of marginal hypothesis with few followers and no funding. However, its rebirth in the 1980s to the present day is based primarily on the application of linearity to estimate cancer risks and to guide costly remediation activities. Many supporters of hormesis have regarded hormesis as proof that the linear model has been, at best, excessively conservative in its low-dose risk estimates or simply wrong. However, although hormesis does directly confront the use of the linear model in cancer risk assessment, its implications are much broader and intriguing because they can be applied to essentially all aspects of biology including evolutionary theory, ecological principles, pharmacology (essentially in the area of drug design, receptor regulation and switching mechanisms) and in the area of chemotherapeutics. As noted above, the present article builds on an earlier article in *TiPS* [1] to address in an overview fashion six additional areas that are crucial to the field of toxicology and are directly impacted by the hormetic dose–response model.

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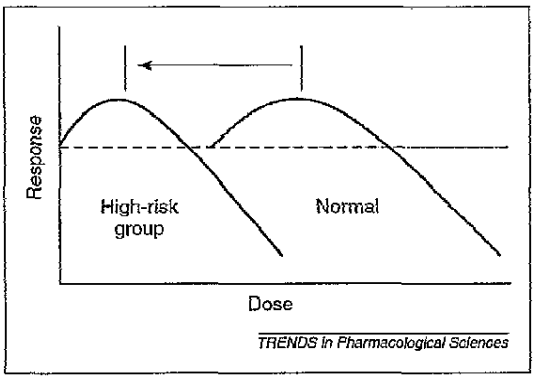

Fig. 1. Stylized dose–response curves illustrating the presence of a hormetic response in high-risk groups. This response occurs at lower doses compared with the response in the normal population and thus the dose–response curve for the high-risk group is shifted to the left of the dose–response curve for the normal population.

Do hormetic effects occur in high-risk groups?

In a recent article by Lave [7] the concept of hormesis was discussed, particularly with respect to its risk assessment implications. Although Lave acknowledged the widespread occurrence of hormetic responses in the toxicological literature, he raised the concern that adaptive responses that underlie the capacity to display hormesis might be lacking in various high-risk groups. This concern was also expressed previously [8]. As a result of these questions and public health concerns an assessment of the developing hormesis database was undertaken to determine whether inter-species and inter-individual differences in susceptibility to toxic substances could be accounted for by the absence of hormetic responses in the more susceptible species and individuals.

The assessment revealed that hormetic dose responses are often present in very susceptible and highly resistant species and individuals [9], which suggests that the causes of differential susceptibility in these instances are independent of the presence of hormetic responsiveness. These findings are significant because they indicate that the hormetic response can occur in the high-risk individual or more sensitive species but at a lower dose. That is, the hormetic dose–response relationship becomes shifted to the left (Fig. 1). In addition, the quantitative features of the dose–response relationship (i.e. the amplitude and width of the low-dose stimulatory response) often appear similar when comparing normal and high-risk groups, but further experimentation is necessary to better define this question.

Despite the above discussion, it is likely that adaptive mechanisms responsible for hormetic responses might be absent within certain high-risk groups. In such cases one would not expect to observe a hormetic response. In fact, research has revealed that the enhanced susceptibility of very young rats to some anticonvulsant drugs is probably a result of the absence of hormetic adaptive responses observed in the more tolerant adult rat (Fig. 2) [10]. In other cases, the presence of hormetic responsiveness was predicted to enhance susceptibility in individuals that displayed a low-dose enhanced cell proliferative response to anti-proliferative drugs designed for cancer chemotherapy [11]. In this case, the presence of the hormetic responsiveness would probably enhance tumor growth (Fig. 3).

The available information therefore indicates a complex pattern of potential responses in which marked differences in inter-species and inter-individual susceptibility can be independent or dependent on the presence of hormesis, as is the case in the enhanced susceptibility to anticonvulsant drugs [11]. Thus, it appears that the concept of hormesis needs to be incorporated into a broadened framework for assessing the basis for inter-species and inter-individual variation.

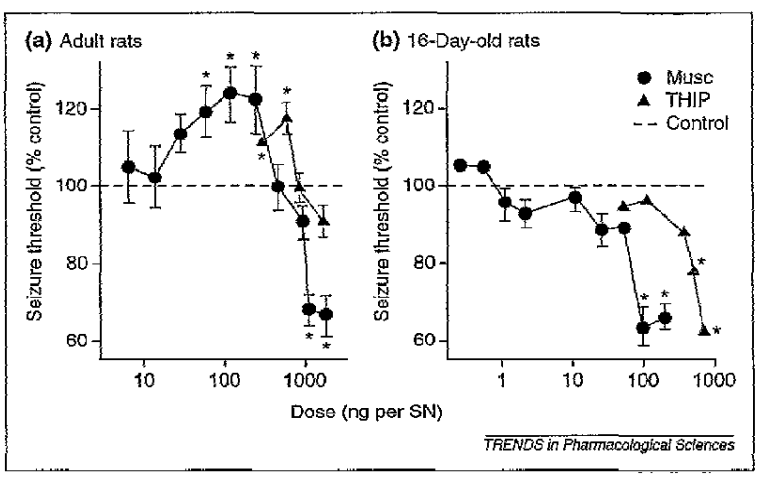

Fig. 2. Dose–response curves for intranigral muscimol and THIP (4,5,6,7-tetrahydroisoxazolo-[5,4c]pyridin-3-ol). Bilateral microinjection of the $GABA_A$ receptor agonists muscimol (musc, purple circles) and THIP (green triangles) into the substantia nigra parts reticulata (SNr) significantly alter flurothyl clonic seizure threshold in adult rats and 16-day-old rats, expressed as a percentage of concurrent saline control response (similar findings for tonic seizure thresholds are not shown). (a) In adult rats the dose–response is biphasic, with intermediate muscimol doses exhibiting a significant dose-dependent anticonvulsant action. High doses of muscimol, however, resulted in a dose-dependent proconvulsant action similar to that of a $GABA_A$ receptor antagonist. The structurally unrelated $GABA_A$ receptor agonist THIP yielded a similar biphasic dose–response pattern, indicating that the proconvulsant effects of high doses of these drugs are most probably a result of a common action on $GABA_A$ receptors in the SNr. (b) In 16-day-old rat pups only proconvulsant effects of intranigral muscimol or THIP were apparent. The expected anticonvulsant effects of $GABA_A$ receptor activation by either agonist were absent in rat pups, indicating an age-related difference in the GABA-mediated response of the SNr. *$P<0.05$, compared with concurrent saline control. Reproduced, with permission, from Ref. 10.

Is there a hormetic mechanism?

Hormesis is a dose–response phenomenon characterized by a low-dose stimulation, high-dose inhibition. This phenomenon occurs independently of species, endpoint and physical or chemical stressor. The broad-based generally applicable nature of this phenomenon suggests the occurrence of a wide range of specific mechanisms that could account for the hormetic response. Numerous documented

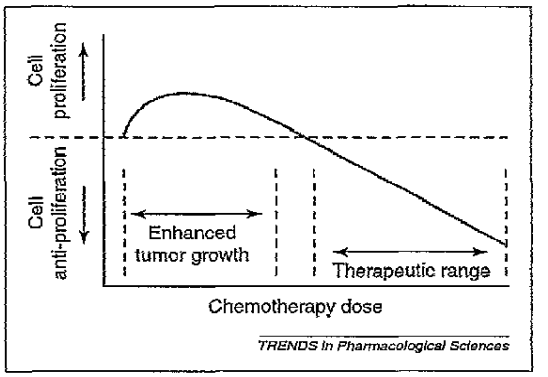

Fig. 3. Stylized dose–response curve illustrating the presence of a hormetic response that enhances susceptibility to tumor growth. Note that this response also applies to viruses and bacteria.

mechanistic explanations have been offered to account for hormetic dose–response relationships. The predominant focus of this mechanistic research accounting for deflection in the dose–response curve (i.e. the hormetic-like biphasic dose–response relationship) has been on receptor-based pharmacological or toxicological responses. To date, hormetic responses within several dozen receptor systems have been elucidated at least to the level of the receptor, and often to levels of further complexity. In many cases a single agonist affects both stimulatory and inhibitory responses as a result differential affinity to receptor subtypes that lead to stimulatory and inhibitory pathways. When such agonists are assessed over a broad range the investigator observes this as the typical hormetic biphasic dose response.

These observations indicate that there is no single hormetic mechanism because such dose responses are mediated via different agonists and receptors depending on the tissue, cell type and endpoint [12] (Fig. 4). The findings suggest that the hormetic phenomenon response is a common, evolutionary-based strategy to carefully regulate resource allocation in a definable range within the context of the re-establishment and maintenance of homeostasis. This conclusion is supported by the fact that the dominant quantitative feature of hormetic dose responses (i.e. low-dose stimulatory responses, regardless of whether it involves a compensatory or directly stimulatory effect) is modest, even at the maximum response.

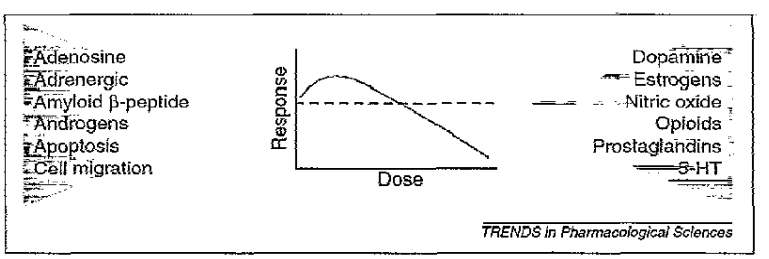

Fig. 4. There is no single mechanism of the hormetic response because this response is mediated by different agonists and receptors, depending on the tissue, cell type and endpoint. Although there are multiple and diverse hormetic mechanisms all or most of such mechanisms appear to be guided by a common evolutionary strategy to maintain or establish homeostasis with minimum resource allocation.

Consistent with the occurrence of a wide range of tissue-specific mechanisms accounting for the hormetic-like biphasic dose–response relationship within a broad resource conservation homeostasis maintenance framework, hormetic dose–response phenomena have remarkably similar quantitative dose–response features. That is, the maximum stimulatory response usually does not exceed two-fold greater than the control value. In general, the maximum stimulatory response is only 30–60% greater than the controls. The range of the stimulatory response is typically less than a factor of 20 with the highest dose being contiguous with the zero equivalent point (i.e. highest dose showing a response equal to the control response). There are instances in which the range of the stimulatory response is far greater than the 20-fold factor, at times exceeding several orders of magnitude of dose (Fig. 5). Explanations to account for the occasionally observed, but highly reproducible, wide stimulatory dose range are considerably under-researched. However, several studies have been published in which manipulation of experimental conditions has greatly modified the range of the stimulatory zone while not affecting the amplitude of the stimulatory response. For example, the dose range of parathyroid-enhanced release of insulin from pancreatic cells was strikingly affected by the concentration of Ca^{2+} within the culture medium [13]. Malnutrition was also shown to affect a change in the dose range of apomorphine-induced heart rate reduction in rats [14]. In the field of experimental psychology, the wide range of the low-dose stimulatory zone can be markedly affected by the level of complexity of the task required [15]. The less complex the task the wider the stimulatory response range and vice versa.

Does hormesis occur in mixtures?

As a general consideration, the concept of hormesis and its relationship to the assessment of mixture toxicology should not be a novel issue. If the hormetic response is considered a fundamental aspect of the dose–response continuum, rather than an unusual or paradoxical phenomenon, its evaluation and interpretation would follow routinely.

The vast majority of experimental data directly relevant to the concept of hormesis has been performed with single agents tested over a broad range of doses. However, hormetic effects have been reported in studies dealing with complex mixtures of petroleum [16–19] and with wastewater effluent [20–22]. Other investigators have reported hormetic responses under better defined conditions of joint or limited multiple exposures [23,24]. These findings clearly indicate that under some defined conditions hormetic effects can occur.

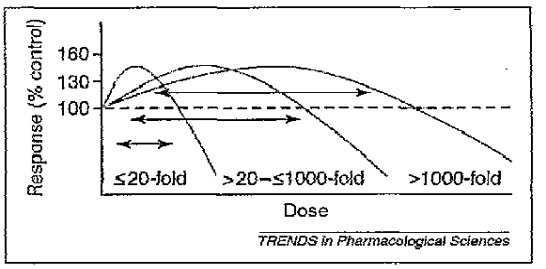

Fig. 5. Stylized dose–response curves illustrating that although the maximum stimulatory hormetic response is generally only 30–60% greater than the control response, the range of the stimulatory response can exceed several orders of magnitude of dose even though it is usually less than 20-fold.

A more detailed consideration of the assessment of common effluents revealed that hormetic-like biphasic dose responses have been routinely observed by US Environmental Protection Agency (EPA) scientists. The quantitative features are similar to the vast majority of hormetic dose responses. In fact, so routinely was the hormetic response observed that the investigators proposed the creation of the term SC20 (i.e. the stimulation concentration for the 20% increase above the controls) to describe the stimulatory response in the low concentration range (Fig. 6) [25].

The assessment of multiple chemical exposures is complicated further by the inclusion of temporal factors. For example, although there is much data to support dose and response additivity under various joint exposure scenarios [26], these relationships often change when one agent is administered before the second. For example, Ewald and Calabrese reported that a prior administration of lead profoundly prevented renal toxicity from a subsequent and more massive exposure to mercuric chloride [27]. Thus, although complex mixtures present formidable challenges to the toxicologist, hormesis should be regarded as an integral and explanatory component of the dose–response relationship within the low-dose segment of the dose–response continuum.

Are temporal features of importance in hormesis?

The issue of temporal features in the assessment of hormetic effects has long been a fundamental basis for confusion over what hormesis is. From an historical perspective it was initially believed that low doses of toxic agents were directly stimulatory of certain biological processes. This concept originated with publications of Hugo Schulz in the 1880s and became embodied within the Arndt–Schulz Law [28,29]. However, this perspective became subsequently challenged by various researchers, particularly in the radiation health effects area, who argued that the stimulatory response occurred only as a compensatory response to previous injury [30,31].

Examination of considerable literature indicates that both sides have sufficient reproducible findings

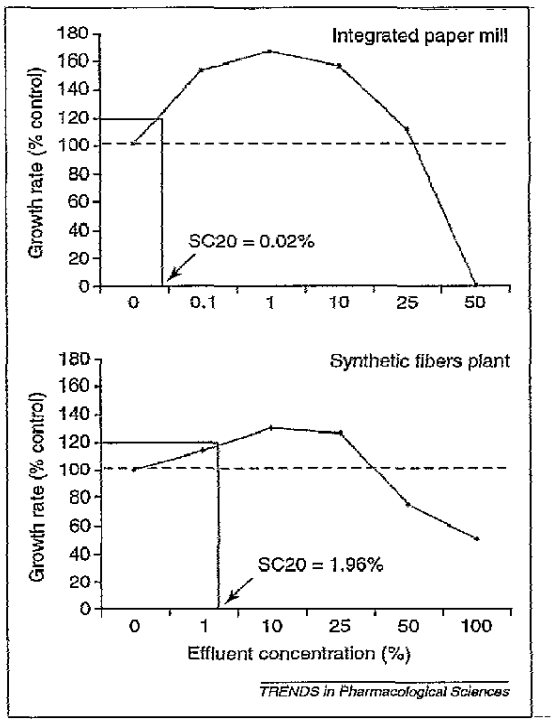

Fig. 6. Representative dose–response curves of common effluents in an integrated paper mill and a synthetic fibres plant. The assessment of common effluents revealed that hormetic-like biphasic dose responses were routinely observed [25]. The quantitative features of the responses were so similar that the investigators proposed the creation of the term SC20, which is the concentration of effluent that elicited a response that was 20% greater than the controls, to describe the stimulatory response in the low concentration range. Data from Ref. 25.

to support the legitimacy of their positions. Thus, hormetic-like biphasic dose responses can occur via either a direct stimulatory response at low doses or via an overcompensation response to an initial disruption in homeostasis. This indicates that the phenomenon of hormesis might result from multiple mechanisms and therefore comprise diverse biological processes. Despite the occurrence of either a direct stimulatory response or an overcompensatory stimulatory response the quantitative features of the dose responses under these very different circumstances are similar. This similarity in dose–response features across an extraordinary range of experimental findings of apparently diverse mechanisms within different tissues and biological models and induced by different stressor agents is an important observation. It suggests the occurrence of an overall regulatory process that limits biological responsiveness within a definable range. Such an overall regulatory strategy appears to be linked to the process of homeostasis, controlling resource allocation. Although the focus of this article is hormetic responses, it is important to recognize that the body has the capacity to respond in certain circumstances

with amplitudes far in excess of the less than twofold widespread hormetic response. Understanding the inter-relationships of these two dose–response phenomena and their control features should be an important consideration.

How does hormesis affect the harmonization of carcinogen and non-carcinogen risk-assessment approaches?

There has been a profound divergence in how carcinogens and non-carcinogens are regulated within the USA. In the case of carcinogens the dose response has been assumed to act linearly at low doses. Non-carcinogens have been assumed to act via a threshold process. This divergence has had an important impact of the derivation of environmental exposure standards particularly for carcinogens when risks are estimated at the 10^{-4}–10^{-6} incidence range. This assumption of a different dose–response relationship for carcinogens and non-carcinogens, which has been a key cornerstone of US regulatory agency risk-assessment procedures for a long time, is inconsistent with the observations emerging from the evaluation of hormetic dose–response relationships. In the case of hormesis the quantitative features of the dose–response relationships are similar regardless of the animal model, tissue affected, endpoint measured (i.e. cancer or non-cancer) and agent tested. Of particular significance is that the nature of the dose–response relationship is similar regardless of the mechanism. These collective observations are of potentially profound theoretical and practical importance affecting issues relating to study design and risk assessment modeling.

What are the medical implications of hormesis?

The recognition of U-shaped dose responses in animal models has been extended to human studies in several instances including those relating to cell culture, clinical and epidemiological investigations. The implications of these inverse dose responses for medical practice are potentially significant. In some instances the U-shaped dose response has been recognized and used in attempts at dose optimization. Below is a brief summary of hormetic responses with clinical applications.

Alzheimer's disease

Several second- and third-generation acetylcholinesterase inhibitors reliably increase cognitive function via inverse U-shaped dose–response relationships in animal models and humans [32–37]. This is just one of many examples of what could be called behavioral hormesis.

Hair growth

Investigators have established that the capacity of minoxidil to enhance hair growth does so in a biphasic manner for multiple parameters [38].

Bone reconstruction

The enhancement of bone production is an important clinical goal in the treatment of osteoporosis and other bone disorders. Basic FGF-2, an endogenous growth factor in bone matrix, has the capacity to stimulate the proliferation of differentiated chondroblasts as well as capillary formation in bone grafts reflecting the low-dose stimulation, high-dose inhibition of the hormetic dose response [39,40].

Tumor suppression and enhancement

Tumors that over-express growth factor genes in tumor tissue have become chemotherapeutic targets via the development of antagonists. Considerable information now indicates that some of these antagonists can act as partial agonists and stimulate cell proliferation at lower doses [11]. These findings have important clinical implications in the treatment of cancer patients.

Viral growth suppression and enhancement

In recent years progress has been made in the development of possible drugs to prevent viral growth, including HIV. It is of interest that some drugs that show promise for therapeutic applications at higher doses also display the potential to enhance viral growth at low doses [41–43].

Immune stimulation

Several external agents, including whole body X-irradiation, at very low doses are known to stimulate various immune functions that have the potential for clinical application based on striking animal findings and preliminary clinical investigations [44–48].

Anti-angiogenetic treatment of tumors

High doses of agents with anti-angiogenetic potential for the treatment of tumors display proliferative responses at lower doses [49]. Such hormetic-like biphasic responses illustrate the crucial need to understand the dose–time–response continuum to develop patient treatment strategies with the highest likelihood of success.

Other implications

Numerous additional hormetic findings of direct relevance to medicinal application have been reported that relate to pain control, sexual functioning, stress, antibiotic effectiveness, guidance for individualizing doses of therapeutic agents, alcohol consumption at low doses, and others.

Concluding remarks

The occurrence of hormetic dose–response relationships in toxicology is widespread, highly

reproducible but dependent on the nature of the study design, animal model selected and endpoint measured. These restrictions have led to the misimpression that hormetic effects are infrequent and even paradoxical. In fact, given an adequate study design [i.e. sufficient number of doses and appropriate dose spacing that ensures that responses at doses below the NOAEL (no observed adverse effect level) are assessed] with sufficient statistical power and appropriate biological model and endpoint selection, hormetic responses become highly predictable [50]. The lack of appreciation of this conclusion is striking particularly for the fields of pharmacology and toxicology where the concept of the dose–response relationship is central to both their theory and practice.

More recent emphasis on the mechanistic understanding of toxicological and/or pharmacological responses has clearly established a family of mechanisms that account for hormetic-like biphasic dose–response relationships [51]. The recognition of the high frequency of hormetic responses in the toxicological and pharmacological literature [50] and the regulatory features of its mechanistic foundations provide a sound basis to support hormesis as a central concept in the biological sciences.

Despite this solid foundation for the concept of hormesis, ~98% of more than 20 000 toxicological articles published over the past 30 years in three toxicologically oriented journals were not designed to assess in a rudimentary way the potential for an hormetic response [50]. This is typically due to the use of a low number of doses and emphasis on greater than NOAEL responses. Because approximately half the articles that do satisfy study criteria for assessing hormesis actually show evidence of it, only 0.5–1.0% of published articles actually demonstrate this phenomenon [50]. This type of reinforcement of irrelevancy continues to encourage the relegation of hormesis to 'paradoxical' status. It is our view that such conditions permit the perpetuation of less persuasive models (i.e. threshold and linear) in governmental risk-assessment activities that affect countless products and standards as well as educational material and instruction at universities regarding toxicology.

Although much remains to be understood about the biology of low doses of chemical, physical, psychological and other stresses, the emerging evidence on hormetic dose responses challenges the long-standing assumption that low-dose responses can be quantitatively and even qualitatively extrapolated from high to low doses with adequate precision and accuracy. The emerging understanding of the fundamentally biphasic nature of the dose–response relationship has the distinct potential to impact current testing protocols for drugs and industrial chemicals and risk-assessment methods that assume linearity at low doses, and identify therapeutic opportunities and previously unanticipated concerns.

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